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SHORT ANALYTICAL REVIEW

Granulocyte-Macrophage Colony-Stimulating Factor: More than a Hemopoietin¹

RODNEY L. MONROY,^{*,2} THOMAS A. DAVIS,^{*} AND THOMAS J. MACVITTIE[†]

**Immunobiology and Transplantation Department, Naval Medical Research Institute, Bethesda, Maryland 20814-5055, and †Department of Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814-5145*

INTRODUCTION

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein with hormone-like properties and naturally occurring molecular mass between 14,500 and 35,000 (1,2). The physical protein structure has been fully described elsewhere (3). Briefly, the base polypeptide has 127 amino acids and its heterogeneity of molecular mass has been attributed to the degree of glycosylation. The glycosylation varies with the cell source but there does not appear to be specific biological roles for the different molecular species (4). Three different forms of the molecule have been cloned, produced in large quantities, and are currently under investigation in clinical trials (5). These recombinant forms are derived from a mammalian cell line (COS cells), bacterial cells (*Escherichia coli*), and yeast, each with varying degrees of glycosylation. The *in vitro* biological properties of these molecules does not appear to be different.

The normal plasma concentration of GM-CSF is below levels detectable by existing techniques (6). It has been shown to be produced in variety of cell types (T-lymphocytes, fibroblasts, stromal cells, dendritic cells, macrophages, and endothelial cells) (2). However, GM-CSF production is induced only in response to a stimulatory signal (e.g., lipopolysaccharide (LPS), interleukin-1 (IL-1), antigen).

The hematopoietic stimulatory activities of GM-CSF *in vitro* have been characterized and the results have been reviewed by others (1, 2, 5, 7, 8). GM-CSF stimulates the proliferation of myeloid, erythroid, and megakaryocyte progenitor cells in the bone marrow cells. While at the other end of the cellular maturation scheme, GM-CSF activates, enhances, and even inhibits functional activities of peripheral blood neutrophils, eosinophils, and monocytes/macrophages. In addition, GM-CSF induces several key inflammatory cytokines: IL-1 (9, 10), tumor necrosis factor (TNF) (11), macrophage colony-stimulating factor (M-CSF) (12), and granulocyte colony-stimulating factor (G-CSF) (13). Therefore, the biological response of an organism to the exogenous addition of GM-CSF is a complex

¹ Views presented in this paper are those of the authors; no endorsement by the Department of Navy or the Defense Nuclear Agency has been given or should be inferred.

² To whom correspondence and reprint requests should be addressed.

interaction of multiple cytokines and cellular responses. This review is organized to present the effects of GM-CSF on normal hematopoiesis and its effect on mature myeloid cells. This will be followed by the results of clinical trials and the future applications of GM-CSF therapy.

EFFECTS ON NORMAL HEMATOPOIESIS

The original research of Metcalf *et al.* (14, 15) first defined the hematopoietic properties of GM-CSF. The purified natural factor stimulated *in vitro* the proliferation of bone marrow granulocyte and/or macrophage progenitor cells to form colonies and also had a specificity to stimulate the more primitive progenitor cells in the bone marrow. The cDNA for GM-CSF has been cloned and its product has the same biological properties as the naturally occurring protein (16). After 14 days in *in vitro*, bone marrow cultures containing GM-CSF formed predominantly monocyte/macrophage colonies with an increased frequency of eosinophil colonies (16). At higher doses of GM-CSF, megakaryocyte colonies were formed (16, 17). In addition, GM-CSF has some erythroid burst-promoting activity (18, 19) and has been shown recently to stimulate a subpopulation of burst-forming units-erythroid (BFU-e) (20). In summary, initial characterization of the hematopoietic stimulatory properties of GM-CSF demonstrates that this factor stimulates early myeloid and erythroid progenitor cells as well as megakaryocytopoiesis. Some of these biological properties of GM-CSF on early hematopoietic progenitor cells overlap with the properties reported for IL-3 (21).

More recent investigations using serum-free *in vitro* culture systems have demonstrated that GM-CSF required secondary factors, G-CSF and M-CSF, to stimulate the formation of colonies (22). The cellular complement to these results was the finding that GM-CSF required "accessory" cells to stimulate colony formation, presumably through the production of the colony-stimulating factors (22). Cellular regulation by GM-CSF apparently increases the number of colonies formed as well as the number of cells per colony (23). A similar condition has been reported for the formation of erythroid colonies (BFU-e) in which both GM-CSF and erythropoietin were required for colony formation *in vitro* (24, 25). This second set of *in vitro* characterizations on the hematopoietic stimulatory properties of GM-CSF suggests that specific cellular or factor requirements are necessary for proliferation of hematopoietic progenitor cells by GM-CSF. This information becomes essential for evaluating the requirements for clinical applications of GM-CSF.

Mice (26, 27), dogs (28), and primates (29, 30) have been used to evaluate the *in vivo* effects of GM-CSF. The most notable change associated with the *in vivo* administration of GM-CSF was an increase in neutrophil counts. In normal animals, continuous or daily infusion of GM-CSF leads to three distinct biological responses, as detected by changes in the peripheral blood neutrophil counts. The first change is a transitory neutropenia (>90% decrease in neutrophil count) which occurs over the first 5 to 120 min of intravenous GM-CSF treatment. This is followed by a neutrophilia which lasts for about 72 hr and is presumably the result of a mobilization of neutrophils from storage pools. A third phase is a further increase in neutrophil counts and occurs after 72-96 hr of GM-CSF treatment. In

this phase, newly formed neutrophils are released from the bone marrow which has been stimulated. Furthermore, an elevated systemic concentration of GM-CSF is required for the maintenance of elevated neutrophil counts since the termination of GM-CSF treatment leads to a decrease in counts, returning to baseline values within 2–3 days. GM-CSF treatment also led to an increase in other peripheral blood leukocytes. Absolute monocyte, eosinophil, and lymphocyte counts are increased in response to treatment. In the primate, the increases in lymphocytes were determined not to be specific for any lymphocyte subpopulation (31).

Although *in vitro* colony data suggested that high doses of GM-CSF stimulated the formation of megakaryocyte colonies, platelet counts in GM-CSF-treated dogs or mice did not increase. In fact, there was a significant reduction (>90% at the highest doses evaluated) in the platelet count of treated dogs (28). However, this dramatic change in the platelets was attributed to a reduction in the platelet half-life, as determined by labeling experiments, with no effect on megakaryocytopoiesis in the bone marrow. In contrast, platelets increased 50–100% in normal primates treated with GM-CSF (32). From these animal studies, the *in vivo* effects of GM-CSF on platelet production are variable. Furthermore, the ability of GM-CSF to stimulate red cell production *in vivo* has not been clearly demonstrated in any of the animal models.

The administration of GM-CSF *in vivo* leads to changes in hematopoietic progenitor cells. A single dose of GM-CSF to mice increased bone marrow myeloid progenitor cells at 72 hr after treatment in a dose-dependent manner. An increase was also observed in the number of progenitor cells in the peripheral blood with a transient peak at 12 hr and a return to baseline by 48 hr. Increases in the peripheral blood myeloid progenitor cells have been attributed to a redistribution of these cells from the bone marrow to the spleen and other organs (liver and lung), where the concentration of myeloid progenitor cells increased (33). In studies involving the daily treatment of dogs (34) or monkeys (32) with GM-CSF, a 50–100% increase in the frequency of bone marrow myeloid progenitor cells has been detected, and activity remained elevated for 7–14 days after termination of treatment.

Human phase I trials using patients with refractory carcinoma and normal bone marrow have provided additional data toward understanding the *in vivo* role of GM-CSF on normal human hematopoiesis. Results of several trials have identified a transient (60–120 min) neutropenia and monocytopenia in the peripheral blood associated with the onset of intravenous GM-CSF treatment (35–37). This was followed by an increase in peripheral blood neutrophils, monocytes, eosinophils, and lymphocytes. No significant changes in either erythrocyte/reticulocyte or platelet counts were observed in these trials. Hence, the administration of GM-CSF to patients has led to results which were comparable to those results obtained from animal models.

The effects of GM-CSF treatment on human myeloid progenitor cells in the bone marrow and peripheral blood have led to several distinct observations. GM-CSF did not have an effect on the frequency of myeloid progenitor cells in the bone marrow (38). However, significant changes in the cell kinetics of myeloid

progenitor cells were detected. The cycling rates of bone marrow progenitor cells was increased (39, 40) and a significant decrease in the cell cycle time (from 86 to 26 hr) was detected in bone marrow of patients treated with GM-CSF (40). In conjunction with the changes observed in the bone marrow, the frequency of myeloid and erythroid progenitor cells in the peripheral blood was increased with GM-CSF treatment. Peripheral blood myeloid progenitor cells increased on the average of 13-fold after 4 to 7 days of GM-CSF treatment (38). The results of these studies suggest that GM-CSF interaction with the bone marrow myeloid progenitor cells is more complex than a simple message to proliferate. Further studies will be required to determine the properties of GM-CSF which regulate the proliferation and release into the peripheral blood of myeloid progenitor cells *in vivo*.

EFFECT ON MATURE MYELOID CELLS

The role for GM-CSF is not limited to the stimulation of hematopoiesis. Metcalf (41) proposed that a primary role of GM-CSF in conjunction with the other colony-stimulating factors (M-CSF and G-CSF) is in the inflammatory response. In his proposal, GM-CSF is produced locally at the site of inflammation by various immune responsive cells (e.g., T-lymphocytes, endothelial cells, macrophages) and its primary purpose is to potentiate the activity of neutrophils to combat infectious organisms. Thus, to understand the biological response to GM-CSF in the clinical setting, it is important to know the regulatory properties of GM-CSF on neutrophils, eosinophils, and monocytes. In this section, the effect of GM-CSF on the mature myeloid end cells will be discussed.

The results of *in vitro* studies on neutrophil function support the premise that GM-CSF is an endogenous regulator of the neutrophil in inflammatory reactions. Neutrophils express GM-CSF receptors on their cell surfaces (42) and the binding of the ligand leads to immediate physical changes. Early reported cellular events are the release of arachidonic acid from membrane phospholipid (43), followed by a modulation of the number and affinity of receptors for the chemotactic peptide *N*-formyl Met-Leu-Phe (fMLP) (44, 45), and an increase in the cell surface glycoproteins associated with the Mo1 complex (37). These cellular events lead to a change in the neutrophil activation status called "priming." The primed neutrophil is hyperresponsive to a second stimulus such as the chemotactic agents: fMLP, soluble products of complement activation (C5a desArg), and arachidonate metabolites, leukotriene B₄ (46). The response of these primed neutrophils to a secondary stimulus leads to an enhancement in oxidative burst (47), membrane depolarization (48), and phagocytosis (49). Thus, GM-CSF prepares the neutrophil for maximum anti-bacterial response.

The immediate decrease in neutrophil counts in response to elevated levels of systemic GM-CSF was identified as neutrophil priming *in vivo*. Within 5 min after initiating intravenous GM-CSF into patients, the neutrophil count was reduced to >90% (37). The decrease was attributed to the expression of a cell surface protein complex (Mo1, CD11b) which was responsible for the adhesion of neutrophils to endothelial cells (50). Neutrophil-labeling studies detected that the neutrophils were sequestered in the lung, presumably by endothelial cells (35). Interestingly, despite continued infusion of GM-CSF, the activation status of circulating neu-

trophils appeared to change with a decreased expression of the Mo1 protein. However, primed neutrophils were still present in the lung associated with endothelial cells and this has potential negative clinical consequences.

The initial results *in vivo* indicate that the effect of GM-CSF on neutrophils *in vivo* was similar to that previously observed in *in vitro* studies. Circulating neutrophils obtained from patients treated with GM-CSF demonstrated a priming of the oxidative burst in response to fMLP (51). Neutrophils obtained from normal monkeys treated with GM-CSF were also primed for oxidative metabolism and bacterial killing (30). These limited results are promising for the use of GM-CSF to enhance neutrophil functions.

A vital property of neutrophils is their ability to migrate from the peripheral blood into tissues and sites of inflammation. The *in vitro* migrational properties of neutrophils are inhibited in the presence of GM-CSF (52). In contrast, GM-CSF had chemotactic properties *in vitro* but only under gradient conditions and at low concentrations of GM-CSF (53). Thus, one can speculate that at an inflammatory site, the local production of GM-CSF would be both an inhibitor of neutrophils within the site and a chemotactic agent to cells distal to the site.

An assessment of the migrational properties of neutrophils exposed to GM-CSF *in vivo*, however, is not clear. Neutrophils of patients treated with GM-CSF did not migrate to sterile skin chambers as well as neutrophils in untreated patients (54). In contrast, neutrophil chemotaxis, when measured *in vitro*, was unchanged in neutrophils obtained from patients in phase I trials (51) and was increased in neutrophils obtained from GM-CSF-treated primates (55). Neutrophil migration is critical and an abnormality in this function increases the risk of infection. Thus, more studies are required to determine what effects elevated levels of systemic GM-CSF have on neutrophil migration *in vivo*. It is possible that continuously elevated levels of GM-CSF have negative effects on neutrophil migration.

Monocyte production is increased by GM-CSF treatment and like neutrophils, peripheral blood monocytes are responsive to the factor. Peripheral blood monocytes treated *in vitro* with GM-CSF have been shown to become cytotoxic against the melanoma cell line A375 (56). This tumoricidal property of monocytes was only previously shown to be induced by two sequential signals, interferon- γ and lipopolysaccharide. In addition, *in vitro* cytotoxicity of monocytes against antibody-coated xenogeneic cells was enhanced in monocytes obtained from patients infused with GM-CSF and the degree of cytotoxicity was increased with the period of infusion (57). Other investigations have reported the *in vitro* activation of monocyte-derived macrophages by GM-CSF for enhanced intracellular killing of *Leishmania tropica* (58). Thus, the cytotoxic properties of monocytes/macrophages are enhanced in the presence of GM-CSF.

The action of GM-CSF on macrophages encompasses not only those in the peripheral blood but also those in the tissues. Macrophages derived from the peritoneal cavity have increased oxidative metabolism and Fc-independent phagocytic activity when incubated *in vitro* with GM-CSF. However, GM-CSF incubation did not increase the degree of Ia expression or the responsiveness of the cells to a challenge by *Toxoplasma* (59). Alveolar macrophages were shown to form colonies *in vitro* in the presence of GM-CSF and their CSF-1-dependent

growth was enhanced by the presence of GM-CSF (60). This demonstrates that GM-CSF has a role in the proliferation of tissue macrophages as well as activating these cells.

Another important biological property of GM-CSF has been its ability to induce secondary factors from monocytes/macrophages. The incubation of GM-CSF with monocytes from normal volunteers induced the expression of the TNF gene (10). However, the *in vitro* secretion of other factors (IL-1, TNF- α , and PGE2) from GM-CSF-treated monocytes has been shown to be dependent upon the costimulator used (61). Furthermore, monocytes obtained from patients treated with GM-CSF and then stimulated *in vitro* with LPS secreted TNF- α and interferon (57). These released cytokines become a part of the biological response to GM-CSF *in vivo*.

The role of GM-CSF in areas of the immune response is under investigation. Several reports have linked GM-CSF as having a role in enhancing the functional expression of antigen-presenting cells and the subsequent augmentation of the primary antibody response (62). A brief treatment of cultured bone marrow macrophages with a pulse of GM-CSF was sufficient to enhance antigen presentation of these cells to a degree superior to cultures in the presence of IFN- γ (63). This enhanced response was attributed to an increase in membrane-bound IL-1 and the induction of Ia molecules. Further studies demonstrated that epidermal Langerhans cells in the presence of GM-CSF mature immunologically to dendritic cells and that this process can be enhanced by the addition of IL-1 (64). These results indicate a role for GM-CSF in the sensitization phase of T-cell-mediated immunity. Other studies have demonstrated that GM-CSF augments the proliferation of T-cells in the presence of IL-2 (65). The role of GM-CSF in the activation or potentiation of other cell types and other arms of the immune response is beginning to be recognized and further investigation should provide information concerning its role in the immune response.

EFFECT ON MYELOSUPPRESSION

GM-CSF has been used in various clinical settings to enhance hematopoietic restoration in patients with myelosuppression. The initial results of clinical trials with hematopoietic growth factors have been recently reviewed (66) and results emphasizing the trials using GM-CSF are presented here.

Studies in myelosuppressed animals have demonstrated that GM-CSF treatment had a significant effect on improving hematopoietic reconstitution (33, 67, 68). Various end points (increased survival; an early recovery of peripheral blood neutrophils; increased number of bone marrow progenitor cells; and increased bone marrow cellularity) have documented an enhancement of hematopoietic recovery. However, primitive bone marrow progenitor cells responsive to GM-CSF are required before the recovery of hematopoiesis can occur. In a recent report, GM-CSF therapy was shown not to be effective until responsive myeloid progenitor cells were present in the bone marrow (68). Therefore, animal studies support the use of GM-CSF therapy for hematopoietic restoration but in addition, a cellular requirement has been identified before hematopoietic recovery can proceed.

Bone marrow transplantation (BMT) models have been used to evaluate the efficacy of GM-CSF treatment on engraftment and neutrophil recovery. Neutrophil recovery after autologous BMT in primates was enhanced with GM-CSF treatment (69, 70). Survival of mice after BMT with suboptimal numbers of syngeneic donor cells was also improved using GM-CSF (71). In a series of allogeneic BMT studies, the timing of GM-CSF treatment was shown to influence the results of the study. *Ex vivo* incubation of T-cell-depleted allogeneic bone marrow cells with GM-CSF enhanced marrow engraftment but survival was not improved over control animals. In contrast, GM-CSF treatment post-BMT improved survival significantly in recipients but engraftment was decreased (72). These results suggest that the timing of GM-CSF treatment must be further evaluated to optimize marrow engraftment as well as to improve recipient survival.

A number of clinical trials have used GM-CSF therapy to enhance hematopoietic reconstitution in patients given autologous BMT (73–75). The results of these trials have demonstrated that GM-CSF treatment accelerated myeloid recovery. In one study (74) there was an improvement in the recovery of platelets over that of historical controls but this has not been reported by others (73, 75). In purged autologous BMTs, a minimal number of transplanted myeloid progenitor cells was required before GM-CSF therapy could enhance neutrophil recovery compared to a group of untreated patients (75). These results suggest that the transplanted myeloid progenitor cell was responsive to the exogenous GM-CSF and after seeding, proliferated to provide an early recovery of neutrophils.

Patients with aplastic anemia have a defect in stem cell proliferation and are pancytopenic. Several trials have evaluated GM-CSF to stimulate myelopoiesis in these patients (76–78). The patients uniformly had severe aplastic anemia and were refractory to anti-lymphocyte globulin. GM-CSF treatment stimulated an increase in neutrophil, monocyte, and eosinophil counts but it had no effect on platelet or red blood cell counts. The elevated counts returned to baseline after termination of treatment. However, even in cases where no substantial rise in the neutrophil count occurred, there was an increase in bone marrow cellularity. This suggested that there was a proliferative response to GM-CSF in the myeloid compartment of the bone marrow. This was further supported by the results of two patients administered repeated cycles of GM-CSF (2 weeks on 2 weeks off) in which baseline neutrophil counts increased with repeated cycles (76). A patient which responded the best to GM-CSF therapy had not received prior therapy before receiving GM-CSF. As discussed in a previous section, GM-CSF may require accessory cells to fully manifest its response and prior treatment may have eliminated these cells. A common conclusion drawn from the reports was that some residual myelopoiesis was required in order for GM-CSF to stimulate an increase in the peripheral blood neutrophil counts.

GM-CSF has also been used to treat patients with myelodysplastic syndrome (MDS), a disease characterized by maturation defects in hematopoiesis which leads to cytopenias and an increased risk of leukemic transformation. Patients with MDS responded favorably to GM-CSF with increases in neutrophils, monocytes, and eosinophils (79–81), and in some cases, treatment led to an increase in platelets (79). However, in one study there was an increase in blast cell counts,

suggesting a stimulation of the leukemic blasts by GM-CSF (81). Thus, the safety of the use of GM-CSF in potential leukemic states must be questioned.

GM-CSF has been used to improve the myelosuppression due to chemotherapy. Phase II trials have demonstrated the ability of GM-CSF to enhance hematopoietic recovery after the first cycle of chemotherapy (82). Further trials are being conducted to increase the number of cycles of GM-CSF and chemotherapy.

Patients infected with human immunodeficiency virus (HIV) and exhibiting the acquired immune deficiency syndrome (AIDS) frequently are neutropenic. Investigations using *in vitro* culture techniques detected an antibody against the envelope glycoprotein (gp120) in the serum of these patients that suppressed the proliferation of myeloid progenitor cells responsive to GM-CSF (83). However, treatment of AIDS patients with GM-CSF led to significant increases in the peripheral blood neutrophil, eosinophil, and monocyte counts (84). Treatment led to the production of neutrophils which had normal functional properties (85) and two of the cases with neutrophil abnormalities were improved. The change in the lymphocyte count was variable from patient to patient and there was no change in the helper to suppressor T-cell ratio. Bone marrow cellularity also improved and remained better after treatment despite a decrease in the peripheral blood counts. Ganciclovir, a virostatic drug, has been used to treat cytomegalovirus infection in AIDS patients but has been shown to induce neutropenia. However, the subcutaneous administration of GM-CSF in conjunction with ganciclovir therapy has led to an improvement in marrow cellularity, peripheral blood counts, and an efficiency of drug therapy (86).

Therefore, the results of clinical trials indicate that in order for GM-CSF therapy to be successful (as determined by improved neutrophil counts), there must exist a certain number of myeloid progenitor cells in the bone marrow. However, as reported in some cases, GM-CSF treatment improved bone marrow cellularity but not the neutrophil counts, suggesting the stimulation of primitive progenitor cells.

The administration of GM-CSF has had some dose-limiting toxicities associated with it. These toxicities include bone pain, fever, edema, rashes, and phlebitis; while at maximum doses, evaluated pericarditis has been reported (66). The mitigation of GM-CSF-induced fever with ibuprofen suggests that some of the toxicities are the result of indirect effects, such as the induction of TNF or IL-1 (87).

FUTURE DIRECTIONS

The biological properties of GM-CSF are extensive and its clinical application, although promising, is less succinct than just as a hematopoietic growth factor. The scope of its role in the inflammatory response, as an example, encompasses many different cell types and includes activation of neutrophils and monocytes at the inflammatory site; recruitment of leukocytes to the inflammatory site; induction of secondary cytokines; potentiation of the functional capacity of antigen-presenting cells; potentiation of the T-cell response; and stimulation of enhanced bone marrow myeloid proliferation. The clinical application of GM-CSF must take into account this broad spectrum of biological activity in developing therapeutic regimens.

Phase II clinical trials using GM-CSF in patients with myelosuppression will provide additional information concerning the ability of this factor, as a single agent, to stimulate primitive hematopoietic cells. Recent investigations have demonstrated that GM-CSF in combination with IL-3 had enhanced hematopoietic stimulatory properties (88). Combinations of hematopoietic growth factors may prove to be more efficient for treating myelosuppression and has promise for patients with aplastic anemia.

The use of GM-CSF in the treatment of AIDS patients has identified that treatment is beneficial for stimulating a neutrophilia. However, *in vitro* results have demonstrated that macrophages infected with HIV-1 had an increased release of HIV particles when incubated in the presence of GM-CSF (89). But a recent report suggests that the combination of AZT and GM-CSF enhanced the efficacy of the drug against virus replication (90). Thus, the possible use of AZT in conjunction with GM-CSF may prove to be beneficial against viral replication as well as to stimulate hematopoietic proliferation.

The ability of GM-CSF to activate and potentiate the functional capacity of neutrophils favors its evaluation in the cases of neutrophil dysfunction (e.g., chronic granulomatous disease or Chediak Higashi disease) where chronic infections are a threat. Burn patients are also susceptible to bacterial infection and exhibit neutrophil dysfunction as well as hematopoietic and immune suppression. It has been demonstrated that a decrease in survival of burn patients was correlated to a decrease in M-CSF (91). Thus, the potential of GM-CSF to override the neutrophil and possibly monocyte defect and stimulate hematopoiesis in burn models needs to be evaluated.

The prophylactic treatment of myelosuppressed mice with GM-CSF was shown to enhance survival against a bacterial challenge (92). The results of clinical trials are too limited to assess the improvement in infection rates in patients treated with GM-CSF compared to untreated controls. However, this aspect of GM-CSF therapy must be actively pursued to determine if such therapy can be used as a clinical management tool against infection.

The potential of GM-CSF in the clinical management of immunosuppression is promising and well-designed experimental studies are required to identify the most appropriate applications.

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